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Use of Salmonella / microsome reversion bioassay for monitoring industrial wastewater treatment plants in Rajasthan, India

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Abstract

Salmonella / microsome reversion assay was used as a biological parameter for monitoring the toxicity of common effluent treatment plant (CETP), Mandia road industrial area, Pali catering to textile industrial areas in Pali, Rajasthan.The influent and effluent water of CETP, surface water (Bandi river) and underground water were tested using Ames bioassay. The results showed presence of mutagens in surface water of Bandi river and the underground water in Pali. Further, comparison of mutagenicity of CETP influent and effluent water revealed that the treatment method employed at this plant has failed to remove mutagenic substances present in Pali textile wastewater. The study also showed that Ames assay is an important tool in genotoxic studies because of its simplicity, sensitivity to genetic damage, speed, low cost of experimentation and small amount of sample required. Further Ames assay, as seen from the results of this study, can be used as a monitoring tool for not only CETPs but also for other water resources. The outcomes of the Ames assay demonstrated its performance as a sensitive, cost-effective and relatively rapid screening tool to assess the genotoxic potential of complex environmental samples.

Key words

Common effluent treatment plant, *Salmonella / microsome* reversion assay, Textile effluents, Mutagenicity

Introduction

Small-scale industries, due to their limited resources in terms of finance, space, and technology, cannot afford to treat their wastes independently. Therefore Central Pollution Control Board (India) in its comprehensive industry document series has advised setting up of common effluent treatment plant (CETP) for small and medium scale dye producing units.

A number of such plants are operational in different parts of India (CPCB, 1990). In Rajasthan three such treatment plants have been established in Pali, Jodhpur and Sumerpur to treat industrial wastes mainly from textile mills. Out of these CETP, Pali has been functional over a long period of time. This plant receives wastewater effluents from 489 textile industries.

Textile industries are known to discharge effluents containing highly toxic compounds (Mathur et al., 2005; Mathur and Bhatnagar, 2007; Hooda, 2007). Chhoakar et al. (2000) characterized the effluents emanating from Pali textile mills and reported high salinity, BOD (400-800 mgl-1) and COD (900-1500 mgl-1), excessive concentration of sodium and carbonate ions; high alkalinity (pH 10.0- 11.5); and low concentrations of calcium in the textile effluents. The results of the study conducted by Mathur et al. (2005) clearly indicate that most of the locally used dyes in Pali are highly mutagenic. The reason for this is that the textile industry is associated with operations like bleaching, mercerizing, dyeing and printing which utilize chemicals like sodium hypochlorite, H_2O_2, acids, surfactants, sodium hydroxide, dyestuffs urea, reducing agents, oxidizing agents, detergents and wetting agents resulting in high alkalinity, salinity, dissolved solids, etc. Further, levels of heavy metals such as

Pb, Cr, Cu and Zn etc., in soils around the industrial area were found to be significantly higher than their normal distribution in soil. High concentrations of these toxic elements in soil were reported to be responsible for the development of toxicity in agriculture products, which in turn effects human life (Krishna and Govil, 2004). Thus, in spite of the CETP, pollution problem in Pali continues to be grave.

Significant variations in the composition of the wastewater arising from a cluster of industries have created difficulties in ensuring the efficiency and effectiveness of the CETP. Besides, one of the major drawbacks of the CETP is that the performance is usually monitored only by physico-chemical parameters such as pH, temperature, oil and grease, suspended solids, BOD and COD. Consideration of only physico-chemical analysis has been thought to be inadequate in protecting the aquatic environment against hazardous discharges (Lambolez *et al*., 1994). Further such analytical monitoring is not enough regarding the potential effects of these effluents on human health. These parameters alone do not reflect genotoxicity or other biological hazards of the effluents. Further, in case of failure of CETP the entire untreated effluent be released to environment. Proper monitoring of industrial treatment plants is thus of utmost importance.

Microorganisms have demonstrated several attributes that make them attractive for use in quick screening of effluents and chemicals for toxicity. Testing of chemicals for mutagenicity in Ames assay is based on the knowledge that a substance that is mutagenic in the bacterium is likely to be a carcinogen in laboratory animals, and thus,by extension, presents a risk of cancer to humans. The Ames test has several advantages over the use of mammals for testing compounds. It is relatively cost effective, only a few days are required for testing a compound and the test is performed with microgram quantities of the material. Such assays are performed on approximately 100 million organisms rather than on a limited number of animals. Therefore to predict the additive, synergistic or antagonistic effect of various chemicals on biological system, bioassay was used. In the light of the above observations, the present work has been planned to use short term microbial assay to monitor the genotoxicity of influent and effluent water from industrial wastewater treatment plants. Further the impact of these industrial wastes on genotoxicity of surface and ground water of Pali was also investigated.

Materials and Methods

Study area: Pali, with a population of 18,19,200 people, is an important district of Rajasthan. It is situated about 70 km from Jodhpur. It has a geographical area of 12,387 sq km and is located between 24.45º to 26.75º N latitude and 72.48º to 74.20º E longitude. Pali, situated on the banks of river Bandi, has got the largest number of textile industries i.e. 989 in the state, mostly engaged in cotton and synthetic textile printing and dyeing.

CETP chosen for the present study was the one installed at Mandia road industrial area, Pali that caters to the need of about 489 textile industries located in this area. This plant treats only industrial waste from these textile dyeing and printing industries. The plant has a capacity to treat 1 million gallons per day (MGD) of wastewater.

Common effluent treatment plant: The plant, developed by Rajasthan Industrial Investment Corporation (RIICO), was commissioned in the year 1986 (CPCB, 2005). National Environment Engineering Research Institute (NEERI) prepared the basic quality of influent and effluent waters. The monitoring parameters are only physicochemical parameters i.e. BOD, COD, pH and total suspended solids.

Sampling: Water samples were collected from five different locations:

Underground water: Two samples were taken, first from a tube well (TU) located in the residential area of Pali while second from the boring water unit inside the effluent treatment plant (BU). Both these sources were being used for drinking water purposes. Surface water: Sample was taken from Bandi nallah drainage that ends up in Bandi river (NL).

CETP: First sample was taken at the point where influent textile wastewaters from the various textile industries at Mandia road, Pali, are entering the treatment plant after passing through the grit chamber (IF). Second sample was taken from the effluent water, which was ready to be discharged into the river (EF). Samples were collected in the month of April and October, which represent the beginnings of summer and winter seasons, and were stored in clean, sterile screw capped glass containers, at 4ºC.

Short- term microbial bioassay: Ames mutagenicity test: All the water samples were tested in the ircrude natural state without concentration The *Salmonella / microsome* reversion assay was conducted using the plate incorporation procedure described by Maron and Ames (1983). TA 98 and TA 100 strains of *S. typhimurium* were obtained from microbial type culture collection and gene bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh (India).

The samples were analyzed with and without the hepatic S9 fraction, which incorporates an important aspect of mammalian metabolism into the *in-vitro* test. Samples were tested on duplicate plates in two independent experiments. Five dose levels of individual samples were tested. Positive controls used without metabolic activation were 2-nitrofluorene for TA 98 (2.5 μg plate^{-1}: 208 revertants) and sodium azide for TA 100 (5 μg plate^{-1}: 2969 revertants). Positive control used with metabolic activation was 2-anthramine for both TA 98 (1 μg plate^{-1}: 481) and TA 100 (1 μg plate^{-1} : 897). Sterile distilled water was used as negative control.

Fresh solutions of the reference mutagens were prepared immediately before the beginning of each experiment. Sterile distilled water was used as negative control (without metabolic activation: - TA 98: 42 revertants and TA 100: 142 revertants; with metabolic activation: TA 98: 44 revertants and TA 100: 168 revertants). All

Table 1 : Mutagenicity ratios of waters from CETP and industrial area, Pali with Salmonella tester strains TA 98 and TA 100

Pali Site	Vol. (ml)	Mutagenicity ratio							
		April				October			
		TA 98		TA 100		TA 98		TA 100	
		- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Boring unit (BU)	2	-	+	-	-	-	+	-	+
	5	+	+	-	-	-	+	-	+
	10	+	+	-	-	+	+	-	+
	50	+	+	-	+	+	+	-	+
	100	+	+	-	+	+	+	-	+
Industrial tube well (TU)	2	-	+	-	-	-	+	-	+
	5	+	+	-	-	+	+	-	+
	10	+	+	-	-	+	+	-	+
	50	+	+	+	+	+	+	-	+
	100	+	+	+	+	+	+	-	+
Drain (NL)	2	+	+	+	-	+	+	-	+
	5	+	+	+	+	+	+	-	+
	10	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Influent CETP (IF)	2	+	-	-	-	+	-	-	-
	5	+	-	+	-	+	-	+	-
	10	+	+	+	-	+	+	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Effluent CETP (EF)	2	+	-	-	-	+	-	+	-
	5	+	+	+	-	+	-	+	-
	10	+	+	+	+	+	-	+	+
	50	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+

+ = Ratio greater than 2.0 indicating mutagenicity, - = Ratio less than 2.0 indicating non- mutagenicity

tester strains were maintained and stored according to the standard methods (Mortelmans and Zeiger, 2000). The strains were regularly checked for genetic markers. All reagents used were of analytical grade, supplied by Himedia Laboratories Limited (India) and Sigma-Aldrich (USA).

Statistical analysis: Multiple post-hoc comparison tests (LSD, Tukey's) were used for statistical analysis. A comparison-wise p-value of <0.05 was considered statistically significant, and all tests were two-tailed. Statistical Package for Social Sciences (SPSS), release 10.0 was used for statistical analysis and graphical representations (Saunders and Trapp, 1990).

Results and Discussion

A number of toxicological studies have been done using algal, plant and animal models (Novotny *et al.*, 2006; Muley *et al.*, 2007) including those on industrial wastewaters (Singh and Singh, 2006; Soni *et al.*, 2006). However, microorganisms have demonstrated several attributes that make them attractive for use in quick screening of effluents and chemicals for toxicity. The use of bioassays is now an essential part of the hazard assessment and control procedures of toxic chemicals and environmental mixtures. In addition to their use in regulatory procedures, bioassays have also been able to assess the performance of wastewater treatment technologies (Jarvis *et al.*, 2006; Alves *et al.*, 2007).

Ames assay has been used extensively in the genotoxicity testing of environmental contaminants. (Ohe *et al.*, 2004). Therefore, using Ames bioassay, influent and effluent water of CETP were examined. Further using the same genotoxicity-based criteria, surface and underground waters of the area surrounding CETP were also analyzed. The results of the Ames test for five different sampling sites are given in Table 1, as the mutagenicity ratio of average induced reversions divided by spontaneous reversions. Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity provided all controls confirm to specifications (Mortelmans and Zeiger, 2000). The mutagenicity tests were made both with and without metabolic activation.

As seen from the mutagenicity ratios, both underground water samples, taken from boring unit and Industrial tube well showed ratios of more than 2.0 indicating positive mutagenicity with strain TA 98 (Table 1). During both the years, there was no significant difference observed in the mutagenic activity in the months of April and October. However, with strain TA 100, the boring water showed

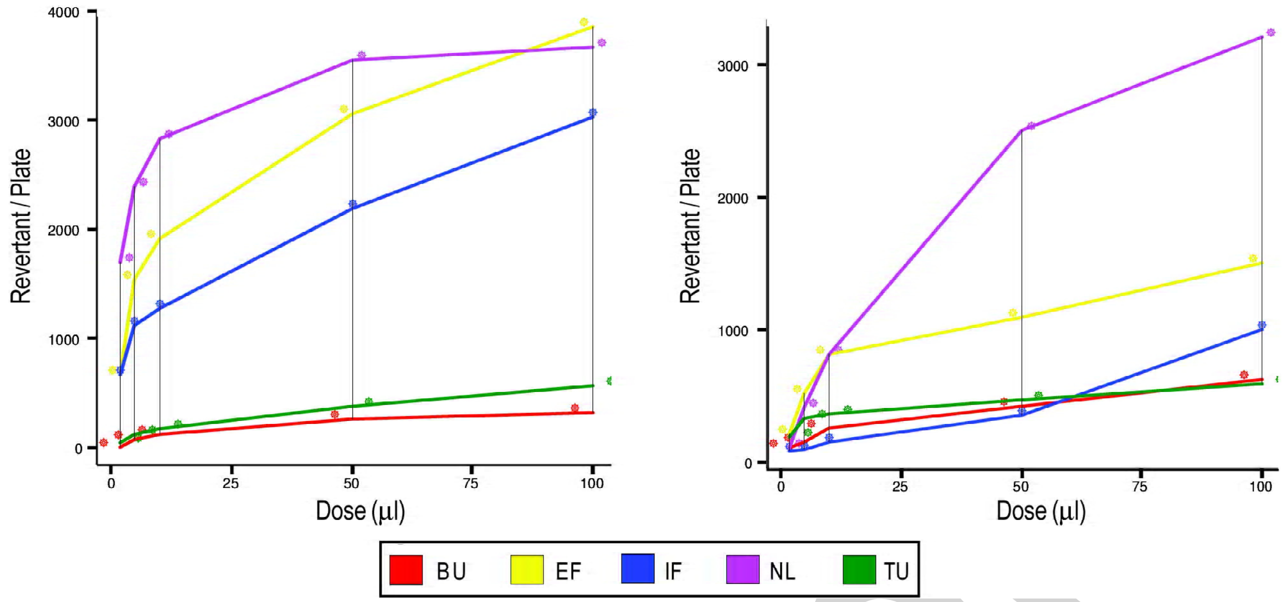

Fig. 1: Dose response curve of water samples of Pali with strain TA 98 of Salmonella typhimurium (April); Error bars show 95.0% CI of mean

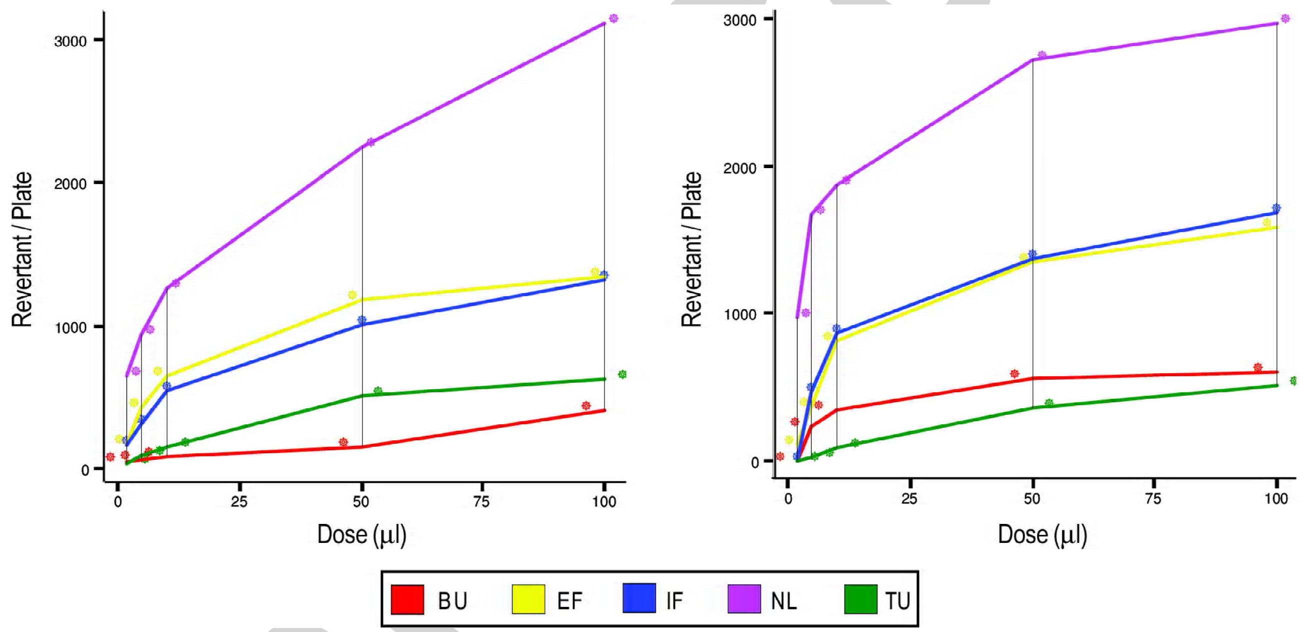

Fig. 2: Dose response curve of water samples of Pali with strain TA 98 of Salmonella typhimurium fraction (October); Error bars show 95.0% CI of mean

complete absence of mutagenicity in the months of April and October.

The second under ground water sample, taken from an industrial tube well, also showed complete absence of mutagenicity during the month of October while during April, positive mutagenicity was seen only at higher doses. Absence of mutagenic response with strain TA 100 is, therefore, indicating absence of base-pair substitution mutagens in these waters. Interestingly, the addition of the hepatic fraction increased the number of revertants, indicating that, mammalian enzymes are possibly converting some of the promutagenic compounds into active mutagenic metabolites.

Further with the surface waters of Nallah drainage, positive mutagenicity was seen from the mutagenicity ratios, for both the strains during April and October and at almost all the dose levels tested. In case of Bandi nallah drainage, the textile industry effluents being discharged were having high mutagenic activity and were mainly responsible for contamination of these surface waters.

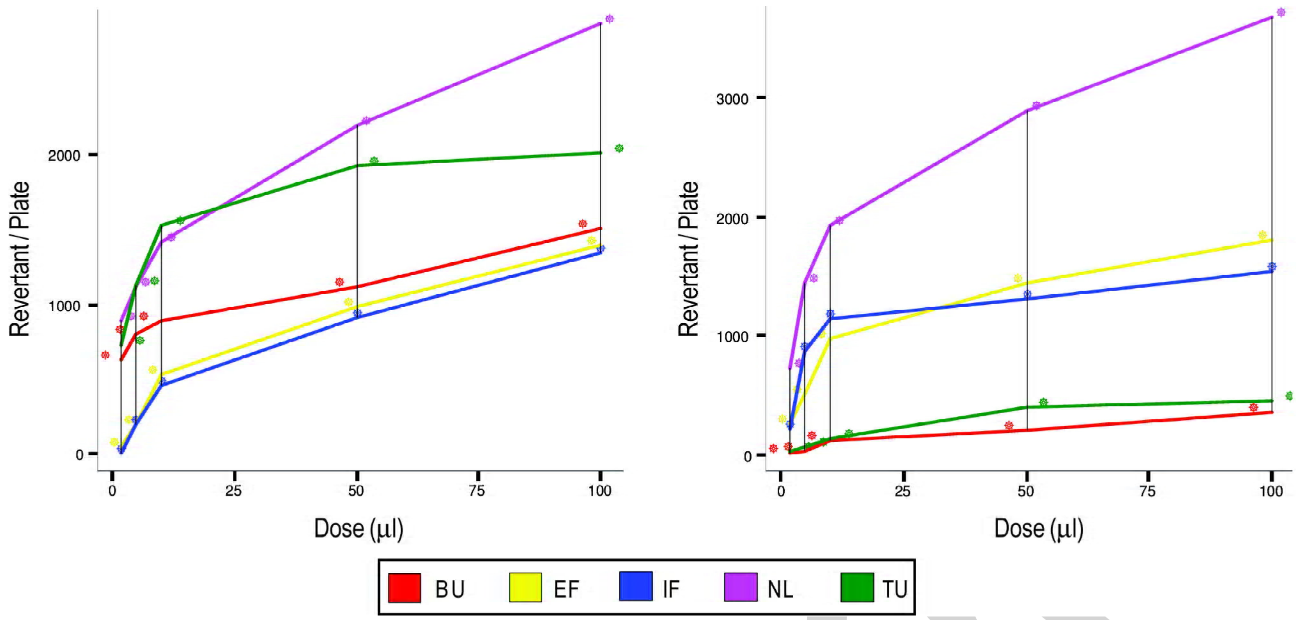

Fig. 3: Dose response curve of water samples of Pali with strain TA 100 of Salmonella typhimurium (April); Error bars show 95.0% CI of mean

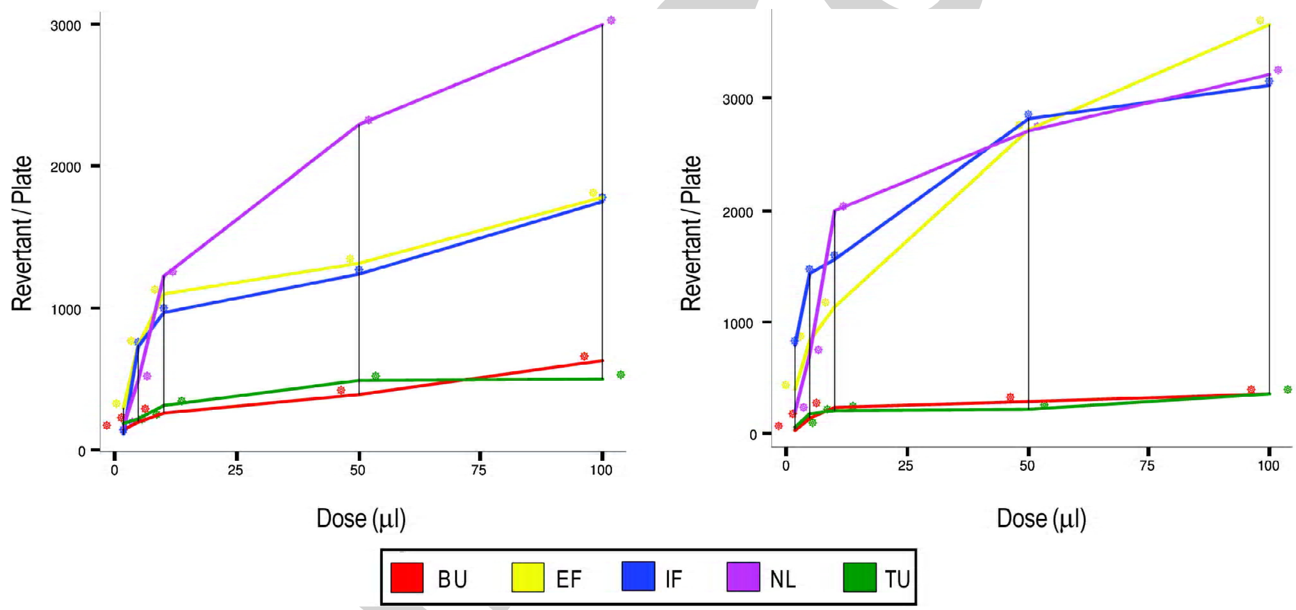

Fig. 4: Dose response curve of water samples of Pali with strain TA 100 of Salmonella typhimurium (October); Error bars show 95.0% CI of mean

More detailed observations were made with the dose response curves of the water samples of the Pali industrial area, shown in Fig. 1 to 4. In case of underground water samples of Pali, with strain TA 98, a clear dose dependent response was obtained, both in presence and absence of S9 hepatic fraction. However, the number of revertants obtained with strain TA 98 during the month of April for boring unit and industrial tube well (345 and 350 induced revertants, per 100 µl of sample, in absence of S9 hepatic fraction, respectively, Fig.1) were similar to those observed during October (320 and 563 induced revertants, per 100 µl of sample, in absence of S9 hepatic fraction, respectively, Fig.2) indicating no significant variation during the months of April and October.

With strain TA 100, during the month of October, a dose dependent increase in the number of induced revertants was obtained but doubling of the number of spontaneous revertants (330 induced revertants, per 100 µl of sterile, distilled water) was not seen even at the highest dose levels tested (320 induced revertants, per 100 µl of sample, Fig.4). When assayed with strain TA 100, during the month of April, boring and tube well waters showed no mutagenicity at lower doses, without S9 mix. However weak, dose dependent mutagenic activity was observed in the presence of S9 hepatic fraction (Fig.3).

Addition of S9 hepatic fraction to underground water samples of Pali industrial area showed significant increase in the number of revertants. With strain TA 98 during April, without S9 mix, boring unit and industrial tube well water had lower number of revertants (345 and 350 induced revertants, per 100 µl of sample, in absence of S9 hepatic fraction, respectively, Fig.1) than with S9 mix or hepatic fraction (628 and 588 induced revertants, per 100 µl of sample, in presence of S9 hepatic fraction, respectively, (Fig.1).

The Bandi nallah drainage water showed high mutagenicity as seen from the number of induced revertants obtained with strain TA 98 and TA 100. No significant difference was observed in the number of induced revertants obtained with strain TA 98 during the months of April (3211 induced revertants, per 100 µ l of sample, in absence of S9 hepatic fraction, Fig.1) and October (3672 induced revertants, per 100 µl of sample, in absence of S9 hepatic fraction, Fig.2). Addition of S9 hepatic fraction to the Nallah water resulted in decrease in the number of revertants obtained. With strain TA 98, lesser numbers of revertants were obtained with S9 hepatic mix, in the months of April and October (3004 and 3216 induced revertants, per 100 µ l of sample, in presence of S9 fraction, respectively, than without S9 fraction (3211 and 3672 induced revertants, per 100 µl of sample, in absence of S9 hepatic fraction, respectively (Fig. 1, 2).

Mutagenic activity of wastewaters of Pali textile dyeing and printing units entering CETP as influent wastewaters (3000-3200 induced TA 98 revertants, per 100 µl of sample, in the absence of S9 liver preparation (Fig. 1, 2) indicates moderate mutagenicity. These results are in agreement with mutagenicity rankings by Houk (1992), which places textile dyeing and textile plant wastewaters in the category of moderate mutagenicity. The net number of TA 98 and TA 100 revertants per liter, in absence of S9 fraction, for wastewaters of Pali textile dyeing and printing units is of the order 106-107 which is almost a degree higher than those reported by Houk (1992) for other textile industry effluents.

Besides the mutagenicity data of the influent wastewater at CETP Pali was comparable to the Bandi drainage Nallah water data. The specific activity of mutagens in the drainage water (3200-3600 induced TA 98 revertants, per 100 µl of sample, in the absence of S9 liver preparation) was of the same order of magnitude as that of mutagens detected in influent wastewater of CETP (3000-3200 induced TA 98 revertants, per 100 µl of sample, in the absence of S9 liver preparation) (Fig. 1, 2).

These results clearly indicate that the textile influent wastewater entering the CETP and the wastewaters being directly discharged in local drainage Nallah contain almost same levels of mutagenic compounds. This is a clear indication that many of the textile dye and printing industries of Pali district are still discharging a large amount of untreated waste directly into local water bodies.

The overall mutagen concentrations in the final effluents being discharged by CETP Pali were no less than those present in the influent wastewater. Influent water of CETP when assayed in the absence of S9 hepatic fraction in the months of April and October (3113 and 3034 induced TA 98 revertants, per 100 µl of the sample, in absence of S9 mix, respectively) showed revertants in the same order of magnitude as the effluent water of CETP (3668 and 3860 induced TA 98 revertants, per 100 µl of sample, in absence of S9 hepatic fraction, respectively). On addition of liver enzyme fraction S9 a comparable decrease in the numbers of revertants was observed for influent water (1745 and 1007 induced revertants, per 100 µl of sample, in, and 1511, induced revertants, per 100 µl of sample, in presence of S9 hepatic fraction, respectively) of CETP, Mandia road, Pali (Fig. 1, 2).

Comparison of the mutagenic response of CETP influent and effluent samples thus revealed that the treatment method employed at this plant has failed to remove certain mutagenic substances, which are present in the Pali textile wastewaters.These results are in agreement with several previous studies which have shown that many of the conventional and advanced wastewater treatment plants have been unsuccessful in adequately removing potentially hazardous chemical mutagens from the wastewater (Rizzo *et al.*, 2009; Gartiser *et al.*, 2010; Torres-Guzman *et al.*, 2010).

Further Ames assay, as seen from the results of this study, can be used as a monitoring tool for not only CETPs but also for other water resources. The outcomes of the ames assay demonstrated its performance as a sensitive, cost-effective and relatively rapid screening tool to assess the genotoxic potential of complex environmental samples.

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